MESOCOTYL AND COLEOPTILE ELONGATION IN SEEDLINGS OF *AVENA SATIVA* AND *TRITICUM AESTIVUM*

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ABSTRACT

Wheat seedlings normally produce no mesocotyl tissue and the growth pattern of the wheat coleoptile is comparable with that of the oat mesocotyl. The mesocotyls of oat and the coleoptiles of wheat seedlings showed an initial rapid growth rate for about 5 days, after which the rate declined and was zero by the ninth day. The coleoptile of oat initially grew more slowly, but entered a phase of rapid growth as the rate of mesocotyl elongation began to decline.

The mesocotyls and coleoptiles of intact seedlings of wheat and oats responded only slightly to exogenously applied IAA and GA_3, while ABA had a strong inhibitory effect.

Isolated segments of tissue of both species responded greatly to IAA and to a lesser extent to GA_3 and ABA.

INTRODUCTION

In dark-grown oat (*Avena sativa*) seedlings both the mesocotyl and coleoptile elongate dramatically in a step-wise pattern (Mer 1951). Initially the mesocotyl elongates rapidly and the coleoptile only begins its rapid growth when the growth of the mesocotyl declines.

Constant light results in the suppression of mesocotyl tissue and the elimination of the early period of slow coleoptile growth (Mer 1951). Intermediate conditions of illumination result in mesocotyls of intermediate length.

Mer (1951) also found that while repeated removal of the coleoptile tip would decrease the growth of the coleoptile, mesocotyl growth was not significantly affected. On the other hand, excision of the coleoptilar node was effective in reducing mesocotyl growth. This led Mer to conclude that growth of the mesocotyl is probably controlled by the coleoptilar node and the plumular growing point, rather than by "auxin" diffusing basipetally from the tip of the coleoptile.

This investigation was concerned with determining the response of coleoptile and mesocotyl tissue of wheat (*Triticum aestivum*) and oats (*Avena sativa*) to exogenously applied hormones. The response of both intact seedlings and isolated segments of tissue was considered and attempts were made to relate any difference in response to current theories on hormonal regulation.

MATERIALS AND METHODS

GENERAL GROWTH PATTERN

Grains of wheat (var. Aotea) and oat (var. Brighton) were sterilised in 0.75% sodium hypochlorite for 5 min., washed thoroughly in distilled water and sown evenly on moist paper in plastic boxes (270 x 200 x 60 mm). The lids were fitted loosely so that aeration was facilitated. Nine boxes of both species were prepared and placed in a darkroom at $25 \pm 1^{o}C$. One box of each species was removed at 24 h intervals, and approximately 50 seedlings from each box were measured (to the nearest mm) to determine mesocotyl and coleoptile lengths. Accurate measurement below 5 mm was difficult, and lengths quoted are estimates.

INTACT SEEDLING EXPERIMENTS

Grains were sterilised and washed as above and sown on 240 mm diameter Whatman No 1 filter paper moistened with distilled water. Filter papers were placed in glass dishes. The dishes were placed in a dark room at $25 \pm 1^{o}C$ and, after 40 h, ten germinated grains were selected and transferred to 90 mm plastic Petri dishes containing Whatman No. 3 filter paper and 7 ml of test solution. Solutions tested were: indole-3-acetic acid (IAA), gibberellic acid (GA), and RS(±) abscisic acid (ABA). Growth in distilled water served as a control. The experiment was set up as a complete randomised block design. Five Petri dishes representing each treatment were placed in plastic boxes (270 x 200 x 60 mm) containing a moist paper towel to prevent dessication. The lid was fitted loosely. Each treatment was replicated four times. All manipulations were carried out under a dim green "safe" light. Grains were incubated in the dark for a further three days, after which mesocotyl and coleoptile lengths were measured to the nearest mm.

IAA and GA_3 were each dissolved directly in distilled water. ABA was dissolved in methanol and dispersed in distilled water to provide a 5×10^{-5} solution containing less than 300 p.p.m. methanol.

SEGMENTS EXPERIMENTS

Grains of oat and wheat were sterilised and sown as outlined above, but were grown in distilled water until the desired seedling height was achieved: oats 40-50 mm, wheat 15-20 mm. This required approximately 4 days. A double-bladed cutting device was used to excise 5 mm mesocotyl segments, 3 mm below the coleoptilar node, from oat seedlings. From both species, 4 mm coleoptile segments were cut, 2 mm behind the apex. Six mesocotyl and coleoptile segments were placed in separate glass Petri dishes (60 mm) containing 8 ml of test solution. Test solutions were buffered with 0.02M phosphate buffer. Growth of segments in buffer solution only was used as a control. Treatments were repeated three times. All manipulations were carried out in dim green light. Segments were incubated in the dark for 24 h, then placed on a glass slide in a photographic enlarger and the magnified image was measured to the nearest mm. Means and standard errors were calculated after allowance for the magnification factor.

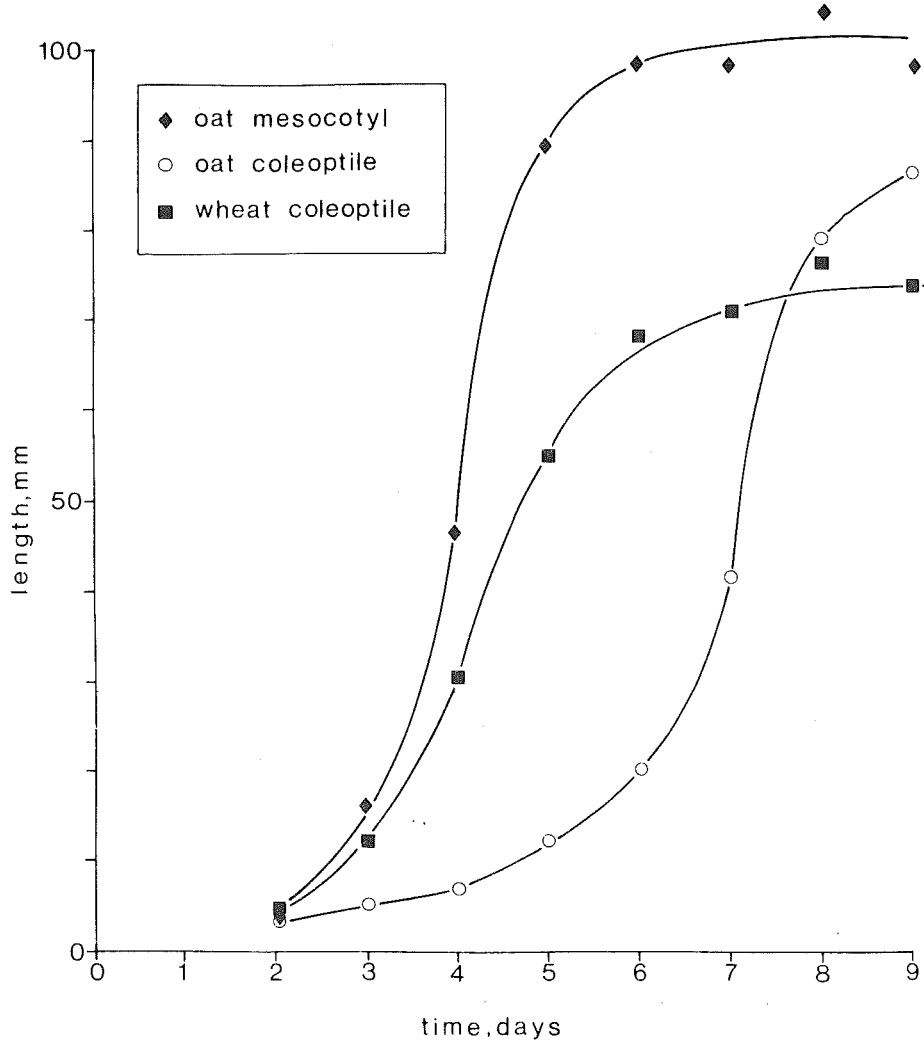

Fig. 1. Elongation of coleoptile and mesocotyl of intact cereal seedlings.

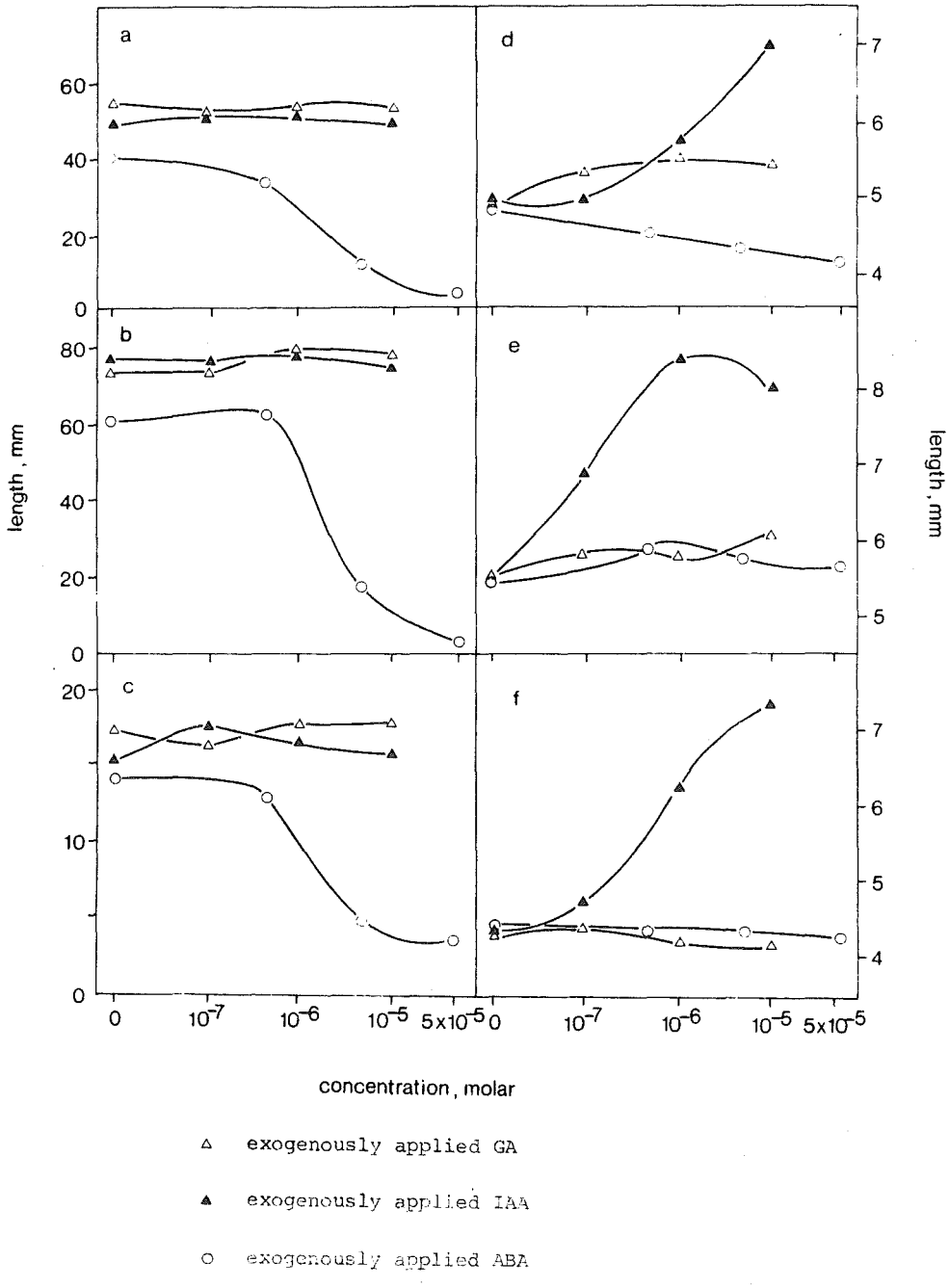

Fig. 2. The response of cereal mesocotyls and coleoptiles to exogenously applied IAA, GA and ABA. Intact seedlings: a, wheat coleoptile; b, oat mesocotyl; c, oat coleoptile. Isolated segments: d, wheat coleoptile; e, oat mesocotyl; f, oat coleoptile.

RESULTS

GENERAL GROWTH PATTERN

The growth patterns of wheat and oat seedlings were determined over a period of nine days (Fig. 1). Oat mesocotyls grew rapidly until day five, when the growth rate declined. Elongation had ceased by the ninth day. The oat coleoptile, however, began growing slowly and entered a phase of rapid growth as the mesocotyl rate began to decline. Growth of the coleoptile also ceased on approximately the ninth day. Final lengths were almost 80 mm for the coleoptile and 100 mm for the mesocotyl.

Wheat seedlings failed to produce mesocotyl tissue, and only a coleoptile was present. This coleoptile behaved essentially like the mesocotyl of oats, and lacked the slow phase of oat coleoptile growth. The final length was approximately 70-80 mm.

INTACT SEEDLINGS

The effect of IAA, GA and ABA on the growth of wheat and oat seedlings is presented in Figs. 2a, b and c. A two-way analysis of variance was carried out to determine the variation between replicates. This was found to be non-significant in all cases. Paired analysis of treatments was undertaken in this section and also in experiments with isolated segments, using the students t-test (2 tailed). Probability levels are given in brackets.

Growth of both oat mesocotyls and coleoptiles (Figs. 2b and c) was almost completely inhibited by 5×10^{-5}M ABA, while 5×10^{-6}M ABA caused a 70% reduction in growth compared with the controls ($P < 0.001$). Lower concentrations had no significant effect on growth. Wheat coleoptiles responded similarly to oat mesocotyls (Fig. 2a) although growth was inhibited by 17% at 5×10^{-5}M ABA ($P < 0.01$).

IAA and GA generally had no promotory effects on either wheat or oat seedling growth. However, oat coleoptile growth was slightly promoted by 10^{-6}M IAA ($P < 0.01$) although this effect was not apparent at higher IAA concentrations. In general, IAA and GA were ineffective in overcoming the initial slow period of growth of oat coleoptiles.

SEGMENT EXPERIMENTS

The response of tissue segments of oats and wheat to 24 h incubation in buffer, IAA, GA and ABA solutions is shown in Figs. 2d, e and f. Oat mesocotyls elongated approximately 0.5 mm in blank buffer, while oat coleoptile segments extended about 0.3 mm. Wheat coleoptile extension, however, was 2-3 times that of oat coleoptiles.

Oat mesocotyl extension was promoted by ABA at all concentrations tested. Maximum promotion occurred at 5×10^{-7}M ABA, where the final length was 10.8% greater in ABA than in buffer solution alone ($P < 0.001$). A significant decrease on the promotive effect occurred at 5×10^{-5}M ABA ($P < 0.01$).

ABA inhibited most of the small amount of extension shown by

wheat coleoptiles in buffer solutions. GA promoted extension of wheat coleoptiles to final lengths 80% greater than those attained in blank buffer. Oat coleoptiles did not respond significantly to either ABA or GA, although oat mesocotyls elongation was promoted by GA. The amount of promotion was very similar to ABA-induced promotion.

IAA promoted elongation of all tissue segments tested. The maximum length achieved by oat mesocotyl segments treated with IAA was 52% greater than the controls. Oat and wheat coleoptiles were 70% and 40% longer respectively. A feature of the oat mesocotyl response to IAA was that the optimum response was achieved at the relatively low concentration of 1×10^{-6}M. No further promotion could be detected at higher IAA concentrations.

Coleoptilar tissue was also very responsive to exogenous IAA. Oat coleoptile segments possessed a greater ability to elongate than did mesocotyl segments. However, oat coleoptiles appeared less sensitive to IAA than did mesocotyl tissue, as little or no promotion of coleoptile tissue occurred at 10^{-7}M IAA.

DISCUSSION AND CONCLUSIONS

The general pattern of growth of oat seedlings conforms to that observed by Mer (1951), although the length of oat mesocotyls reported in this paper is slightly greater. Wheat, which lacks a mesocotyl, has coleoptile growth similar to the oat mesocotyl pattern and lacks the initial slow phase of growth. Further study could be carried out to determine whether or not initials for mesocotyl tissue are present. The inverse relationship of the growth of mesocotyl and coleoptile tissue of oat, shown in Fig. 1, where the coleoptile increases its rate of growth only as the mesocotyl ceases its initial rapid growth, is possibly due to auxin-directed transport, which creates a "metabolic sink" in the mesocotyl tissue. The probable sites of control of this process are the coleoptile tip and the coleoptilar node as postulated by Mer (1951). He reported that while local illumination of the coleoptile tip produced an effect on mesocotyl growth, local illumination of the coleoptilar node produced a far greater reduction. Also, removal of the coleoptile tip did not greatly effect mesocotyl growth, and where an effect was found it could be associated with a wound reaction.

Mer (1951) considered it unlikely that auxin diffusing from the tip of the coleoptile controlled mesocotyl growth, for when the mesocotyl is growing at its maximum rate, coleoptile growth is slow, despite the fact that the auxin transmitted to the mesocotyl must, on this hypothesis, have passed through the coleoptile tissues. However, in this report, and that of Thimann (1969), isolated segments of coleoptile were found to be less sensitive to IAA than were mesocotyls. Whether this differential response to IAA would, alone, be sufficient to explain the control of mesocotyl and coleoptile growth by auxin is debatable. In the light of the evidence above it would appear that both the coleoptilar node and coleoptilar tip may be important in the control of seedling growth.

Dark-grown seedlings undergo a process known as etiolation; the plant uses the limited supply of storage materials predominantly for axis growth. In this way the plant endeavours to carry the tip of the plant to any possible source of light before the storage material is exhausted. Under these conditions it would be reasonable to expect the plant to synthesise optimal amounts of stimulatory hormones in order to reach its goal. Any exogenous application of promotory hormones would create supra-optimal concentrations of those hormones within the plant, and therefore the plant could well be expected to have some mechanism by which it can maintain its desired hormone level. Plant do, in fact, have such mechanisms: IAA can be degraded enzymatically, GA can be converted to inactive neutral, or bound forms (Thimann 1969), and ABA has 5 known metabolites (McWha and Hillman 1972, Sondheimer *et al.*1974).

Isolated segments of mesocotyl and coleoptile are particularly sensitive to exogenously applied IAA and this suggests that IAA may be of particular importance in etiolation. While not necessarily the only hormone involved, as GA has slight promotory properties in isolated segments, IAA appears to be the most important of those tested.

The effects of plant hormones on wheat coleoptile segments found in this report are consistent with those found by Barlow, *et al.* (1961). The promotion of oat coleoptile extension by IAA and the inhibition by ABA has previously been reported by Thomas *et al.* (1965) and Philipson *et al.* (1973). The promotion of oat mesocotyl segments by IAA and GA reported here is similar to that found by Milborrow (1966). The observed promotive effect of ABA on isolated oat mesocotyl tissue is, however, contrary to the findings of Milborrow (1966). He found ANA had no stimulatory effect on oat mesocotyls, although ABA stimulation of intact rice mesocotyls has been reported by Takahashi (1972, 1973). No interpretation of this ABA promotion of oat mesocotyl can be made without further work.

Milborrow (1966) also claimed that while GA is not normally synthesised in mesocotyls, IAA is, and that the small amount of elongation of mesocotyls tissue in blank buffer is caused by endogenous IAA. Consideration must also be given to wounding effects which can result in increased rates of growth, depending on the severity of treatment (Idle 1955).

It is apparent from this investigation that growth of intact oat coleoptiles is somehow held in check, for, once isolated, segments respond greatly to exogenously applied IAA and to a lesser extent GA.

Why then do isolated segments of mesocotyl and coleoptile respond strongly to added promotive hormones, when applications to intact plants have little effect? One possibility is that in intact plants the sites of action of IAA are saturated, and therefore exogenously applied IAA can have no effect. In isolated segments, however, the source of IAA is probably reduced. Therefore sites of action become available to added IAA. This, however, does not explain why oat coleoptiles are not stimulated by exogenous IAA, because at the stage of rapid mesocotyl growth they have yet to undergo their period of maximum extension. Another possible

answer is that metabolic break-down of exogenous IAA in isolated segments is reduced due to the removal of the controlling centre of extension or the site of hormone breakdown. It is possible that these sites are one and the same; probably the coleoptile tip and/or the coleoptilar node. Also, the possible role of the roots or the seed in metabolising unwanted hormones cannot be overlooked.

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